

THE CLAIMS

What is Claimed is:

- 5 1. A rumen bacterial mutant which a lactate dehydrogenase-encoding gene (*ldhA*) and a pyruvate formate-lyase-encoding gene (*pfl*) have been disrupted, and has the property of producing succinic acid at high concentration while producing little or no other organic acids in anaerobic conditions.
- 10 2. A rumen bacterial mutant which a lactate dehydrogenase-encoding gene (*ldhA*), a pyruvate formate-lyase-encoding gene (*pfl*), a phosphotransacetylase-encoding gene (*pta*) and a acetate kinase-encoding gene (*ackA*) have been disrupted, and has the property of producing succinic acid at high concentration while producing little or no other organic acids in anaerobic conditions.
- 15 3. A rumen bacterial mutant which a lactate dehydrogenase-encoding gene (*ldhA*), a pyruvate formate-lyase-encoding gene (*pfl*), and a phosphoenolpyruvate carboxylase-encoding gene (*ppc*) have been disrupted, and has the property of producing succinic acid at high concentration while producing little or no other
- 20 organic acids in anaerobic conditions.
4. The rumen bacterial mutant according to any one claim among claims 1-3, wherein the rumen bacteria are selected from the group consisting of genus *Mannheimia*, genus *Actinobacillus* and genus *Anaerobiospirillum*.
- 25 5. The rumen bacterial mutant according to any one claim among claims 1-3, wherein the rumen bacteria are homo-fermentative bacteria that produce only succinic acid while producing little or no other organic acids.
- 30 6. The rumen bacterial mutant according to claim 1, wherein the rumen bacterial

mutant is *Mannheimia* sp. LPK.

7. The rumen bacterial mutant according to claim 6, wherein said *Mannheimia* sp. LPK is KCTC 10558BP.

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8. The rumen bacterial mutant according to claim 2, wherein the rumen bacterial mutant is *Mannheimia* sp. LPK7.

9. The rumen bacterial mutant according to claim 8, wherein said *Mannheimia* sp. LPK7 is KCTC 10626BP.

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10. The rumen bacterial mutant according to claim 3, wherein the rumen bacterial mutant is *Mannheimia* sp. LPK4.

11. A method for producing rumen bacterial mutant that has the property of producing succinic acid at high concentration while producing little or no other organic acids in anaerobic conditions, the method comprising disrupting a lactate dehydrogenase-encoding gene (*ldhA*) and a pyruvate formate-lyase-encoding gene (*pfl*) from rumen bacteria that are selected from the group consisting of genus *Mannheimia*, genus *Actinobacillus* and genus *Anaerobiospirillum*.

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12. A method for producing rumen bacterial mutant that has the property of producing succinic acid at high concentration while producing little or no other organic acids in anaerobic conditions, the method comprising additionally disrupting a phosphotransacetylase-encoding gene (*pta*) and an acetate kinase-encoding gene (*ackA*) from rumen bacteria that are selected from the group consisting of genus *Mannheimia*, genus *Actinobacillus* and genus *Anaerobiospirillum*, and a lactate dehydrogenase-encoding gene (*ldhA*) and a pyruvate formate-lyase-encoding gene (*pfl*) have been disrupted.

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13. A method for producing rumen bacterial mutant that has the property of producing succinic acid at high concentration while producing little or no other organic acids in anaerobic conditions, the method comprising additionally disrupting a phosphoenolpyruvate carboxylase-encoding gene (*ppc*) from rumen
5 bacteria that are selected from the group consisting of genus *Mannheimia*, genus *Actinobacillus* and genus *Anaerobiospirillum*, and a lactate dehydrogenase-encoding gene (*ldhA*) and a pyruvate formate-lyase-encoding gene (*pfl*) have been disrupted.
- 10 14. The method for producing the rumen bacterial mutant according to claim 12 or 13, wherein the rumen bacterial mutant having disruptions of a lactate dehydrogenase-encoding gene (*ldhA*) and a pyruvate formate-lyase-encoding gene (*pfl*) is *Mannheimia* sp. LPK (KCTC 10558BP).
- 15 15. The method for producing the rumen bacterial mutant according to claim 11, wherein the disruption of the *ldhA* and *pfl* genes is performed by homologous recombination.
16. The method for producing the rumen bacterial mutant according to claim 15,
20 wherein the homologous recombination is performed using a genetic exchange vector containing a disrupted *ldhA* and a genetic exchange vector containing a disrupted *pfl*.
17. The method for producing the rumen bacterial mutant according to claim 16,
25 wherein the genetic exchange vector containing a disrupted *ldhA* is pMLKO-sacB, and the genetic exchange vector containing a disrupted *pfl* is pMPKO-sacB.
18. The method for producing the rumen bacterial mutant according to claim 12,
30 wherein the disruption of the *pta* and *ackA* genes is performed by homologous recombination.

19. The method for producing the rumen bacterial mutant according to claim 18, wherein the homologous recombination is performed using a genetic exchange vector containing a disrupted *pta* and *ackA*.

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20. The method for producing the rumen bacterial mutant according to claim 19, wherein the genetic exchange vector containing a disrupted *pta* and *ackA* is pPTA-sacB.

10 21. The method for producing the rumen bacterial mutant according to claim 13, wherein the disruption of the *ppc* gene is performed by homologous recombination.

15 22. The method for producing the rumen bacterial mutant according to claim 21, wherein the homologous recombination is performed using a genetic exchange vector containing a disrupted *ppc*.

23. The method for producing the rumen bacterial mutant according to claim 22, wherein the genetic exchange vector containing a disrupted *ppc* is pPPC-sacB.

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24. A genetic exchange vector pMLKO-sacB containing a disrupted *ldhA*.

25. A genetic exchange vector pMPKO-sacB containing a disrupted *pfl*.

25 26. A genetic exchange pPTA-sacB containing a disrupted *pta* and *ackA*.

27. A genetic exchange vector pPPC-sacB containing a disrupted *ppc*.

28. A method for producing succinic acid, the method comprising the steps of: culturing the rumen bacterial mutant of any one claim among claims 1-3 in anaerobic condition; and recovering succinic acid from the culture broth.
- 5 29. The method for producing succinic acid according to claim 28, wherein the culturing step is homo-fermentation which produce succinic acid at high concentration while producing little or no other organic acids.
30. The method for producing succinic acid according to claim 28, wherein the
10 rumen bacterial mutant is *Mannheimia* sp. LPK, LPK7 or LPK 4.